Role of catalase on the hypoxia/reoxygenation stress in the hypoxia-tolerant Nile tilapia

Alexis F. Welker,1,2 Élida G. Campos,1 Luciano A. Cardoso,1 and Marcelo Hermes-Lima1

1Laboratório de Radicais Livres, Departamento de Biologia Celular, and 2Faculdade da Ceilândia, Universidade de Brasília, Brasília, Brazil

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Welker AF, Campos ÉG, Cardoso LA, Hermes-Lima M. Role of catalase on the hypoxia/reoxygenation stress in the hypoxia-tolerant Nile tilapia. Am J Physiol Regul Integr Comp Physiol 302: R000–R000, 2012. First published February 29, 2012; doi:10.1152/ajpregu.00243.2011.—The specific contribution of each antioxidant enzyme to protection against the reoxygenation-associated oxidative stress after periods of hypoxia is not well understood. We assessed the physiological role of catalase during posthypoxic reoxygenation by the combination of two approaches. First, catalase activity in Nile tilapia (Oreochromis niloticus) was 90% suppressed by intraperitoneal injection of 3-amino-1,2,4-triazole (ATZ, 1g/kg). In ATZ-injected fish, liver GSH levels, oxidative stress markers, and activities of other antioxidant enzymes remained unchanged. Second, animals with depleted catalase activity (or those saline-injected) were subjected to a cycle of severe hypoxia (dissolved O2 = 0.28 mg/l for 3 h) followed by reoxygenation (0.5 to 24 h). Hypoxia did not induce changes in the above-mentioned parameters, either in saline- or in ATZ-injected animals. Reoxygenation increased superoxide dismutase activity in saline-injected fish, whose levels were similar to ATZ-injected animals. The activities of glutathione S-transferase, selenium-dependent glutathione peroxidase, and total-GPX and the levels of GSH-eq, GSSG, and thiobarbituric acid reactive substances remained unchanged during reoxygenation in both saline- and ATZ-injected fish. The GSSG/GSH-eq ratio in ATZ-injected fish increased at 30 min of reoxygenation compared with saline-injected ones. Reoxygenation also increased carbonyl protein levels in saline-injected fish, whose levels were similar to the ATZ-injected group. Our work shows that inhibition of liver tilapia catalase causes a redox imbalance during reoxygenation, which is insufficient to induce further oxidative stress. This indicates the relevance of hepatic catalase for hypoxia/reoxygenation stress in tilapia fish.

aminotriazole; glutathione peroxidase; anoxia; reactive oxygen species; antioxidant

Tissue exposure to ischemia and subsequently to hypoxia/anoxia can cause cell damage and death in most animals. In rats, for example, during a short ischemic event, a large fraction of the myocardium remains intact; however, lesions and necrosis arise upon reperfusion (58). It is well recognized that ischemia-derived injury is greatly increased during reoxygenation. In isolated rat hearts, after complete ischemia for 45 min, reperfusion with a normoxic solution results in a more intense degree of tissue damage than reperfusion with a hypoxic solution (47). Studies with whole animals also show loss of vital functions due to drastic variations in oxygen availability. Flies submitted to repeated anoxia and reperfusion episodes have their spiracular control system damaged, causing dehydration and death (27).

Cellular damage occurring upon reoxygenation may be due to an overproduction of reactive oxygen species (ROS) (23, 51). ROS function as intracellular signals, but can be deleterious when produced in excess. Antioxidant enzymes play an important role in the removal of ROS and, therefore, can have significant effects in reducing the oxidative damage during reoxygenation. Hearts of mice overexpressing the antioxidant enzyme superoxide dismutase (SOD) present reduced infarct size associated with increased superoxide radical removal after an ischemia-reperfusion event compared with nontransgenic controls (59). In addition, induction of catalase, Mn-SOD, and Cu-ZnSOD during renal preconditioning to ischemia is involved in the protection of the rat kidney to subsequent ischemia/reperfusion injury (61). These results indicate that reoxygenation-induced oxidative stress can be significantly inhibited by the modulation of one or more antioxidant enzymes. The importance of any particular antioxidant enzyme for the reoxygenation protection has not been established (62).

There are several animals that naturally deal with abrupt fluctuations in oxygen availability without having to face the danger of excessive oxidative stress. The high tolerance to hypoxia observed in various ectotherms makes them suitable models to study the capacity for surviving adverse conditions (7). Some of these animals modulate the antioxidant system before reoxygenation by increasing the activity of different antioxidant enzymes, avoiding oxidative stress or maintaining it at a physiologically manageable level (13, 23). For example, an increase in the activity of catalase takes place in the liver of goldfish during a 8-h anoxia exposure, suggesting a possible role of this enzyme in minimizing postanoxic oxidative stress (32). Similar increases in the activity or expression of antioxidant enzymes were observed in different hypoxia-tolerant animal species in response to hypoxia/anoxia (11, 20, 22, 31, 32, 52). Normally, these animals show an impressive drop in metabolic rate, diminishing the demand of oxygen and energy reserves (7). During metabolic depression, especially when there is a fall in oxygen supply (26, 40), there is a general drop in the rate of protein synthesis, a costly process in terms of ATP consumption. Therefore, the increased expression of antioxidant enzymes during hypometabolic states, at first glance, seems to be counter-productive. However, several studies indicate that it may play an efficient protective role against oxidative stress (13, 23).

The understanding of the relevance of each one of the antioxidant enzymes could reveal which mechanism is primarily responsible for the high resistance that hypoxia-tolerant animals have against reoxygenation stress. Few researchers have investigated the role of a specific antioxidant enzyme by means of inhibiting its activity in vivo, and these studies were conducted under constant levels of oxygen concentration, pH,
and temperature. Bagnyukova et al. (2, 3) investigated the role of catalase in the redox homeostasis of goldfish by employing injections of the catalase inhibitor 3-amino-1,2,4-triazole (ATZ) (43)—in this case, the animals were maintained under normoxia. Consequently, the data generated in such studies do not explain the physiological relevance of a particular antioxidant enzyme during hypoxic/anoxic challenges. Therefore, the aim of the present study was to assess the role of catalase on protection against reoxygenation stress in the Nile tilapia fish (*Oreochromis niloticus*) by inhibiting its activity in vivo using ATZ.

**Materials and Methods**

**Chemicals.** All reagents used were of analytical grade and are listed in a previous publication (45). All solutions were prepared with Milli-Q deionized water.

**Animals and acclimation conditions.** Male young adult Nile tilapia (*Oreochromis niloticus*) weighing 80–160 g were purchased from a fish farm (Psicultura Retiro, 300 km from Brasilia, Brazil). Before any experimental procedure, animals were maintained in the laboratory during an acclimation period for 3 wk. The animals were kept in 200-liter tanks with filtered and dechlorinated water (a maximum of 15 individuals per tank), with controlled photoperiod (12:12-light-dark cycle), temperature (22–25°C), and daily monitored pH and ammonia levels. We used coal filters and switched half of the water every 1–2 days. The tanks were constantly bubbled with air. Animals were fed with standard fish food. The animal-handling and euthanasia method used in this study complied with the ethical principles in animal testing described by the Brazilian Federal Law (11,794/2008) and were in accordance with the Guide for Care and Use of Laboratory Animals (www.nap.edu). The experiments reported, herein, were performed in 2003–2004.

**Preliminary tests to standardize ATZ dosage.** Previous ATZ dose used for in vivo studies in nonmammals is 1 g/kg body mass (2, 3). To analyze whether this dosage causes a significant reduction in catalase activity in tilapia without causing mortality, animals were injected intraperitoneally with ATZ diluted in saline 0.9% and were in accordance with the Guide for Care and Use of Laboratory Animals (46). The activities of catalase, glutathione S-transferase (GST), glutathione reductase (GR), and selenium-dependent glutathione peroxidase (Se-GPX) in liver homogenates were measured following procedures described by Ramos-Vasconcelos et al. (44). Briefly, catalase activity was determined by following the rate of H$_2$O$_2$ decomposition; GST activity was measured by following the rate of GSH reaction with 1-chloro-2,4-dinitrobenzene; GR and Se-GPX (using H$_2$O$_2$ at a final concentration of 0.2 mM), activities were measured by following the rate of NADPH oxidation. To measure the activity of total-GPX, the test conditions were the same as those used to measure Se-dependent GPX, as described by Ramos-Vasconcelos et al. (44), except that H$_2$O$_2$ was replaced by cumene hydroperoxide at a final concentration of 2 mM.

Total SOD activity was measured by monitoring the SOD-induced inhibition of nitroblue tetrazolium reduction to formazan by superoxide radicals (5) under the following assay conditions: 0.1 mM EDTA, 0.025 mM nitroblue tetrazolium, 0.1 mM hypoxanthine, and 30 mM/xantine oxidase in 50 mM Na$_2$CO$_3$ buffer, at pH 10. The rate of increase in absorbance was recorded for 3 min at 560 nm. One unit of SOD was defined as the quantity of SOD required to produce 50% of the maximum inhibitory capacity of SOD in the assay.

**ATZ concentration in liver.** Homogenates were obtained as described in Antioxidant enzymes. They were mixed with trichloroacetic acid 10% (1:1) and centrifuged at 2,700 g. One hundred microliters of the supernatants were mixed with 20 μl of 10 mM nitrous acid (diazotizes ATZ, produces diazide from amine). Diazoitized ATZ was then coupled with 2.5 mM 4-dihydroxynaphthalene-2,7-disulfonic acid (chromotopic acid) by adding 20 μl of this reagent to the samples. The samples (140 μl total) were then heated at 100°C for 2.5 min to form a colored derivative, which was read at 525 nm (14, 43).

**Determination of glutathione, lipid peroxidation, and carbonyl protein.** Frozen tissue samples were homogenized (1:10 wt/vol) in ice-cold 5% sulfosalicylic acid and centrifuged at 15,000 g for 5 min. Supernatants were used to measure glutathione levels, as described previously (45).

**Protein measurements and statistics.** Protein concentration was measured by the modified method of Lowry (18), using BSA as a standard. All values were expressed as means ± SE. The results were analyzed by one-way ANOVA, followed by a Dunnett’s test. For comparisons between saline- and ATZ-injected animals during the different moments of reoxygenation, we applied the step-down Bonferroni-Holm adjustment to correct for multiple comparisons after reoxygenation. Groups of 6 or 7 individuals were killed by decapitation at six time points: 3 h after hypoxia condition and 0.5 h, 2 h, 6 h, 12 h, and 24 h after reoxygenation. In all cases, the liver was quickly removed, frozen in liquid nitrogen, and stored at −75°C for biochemical analysis.
free radical metabolism, leading to changes in other antioxidant enzymes. Therefore, antioxidant enzyme levels from animals subjected to ATZ/normoxia or hypoxia alone were compared with data from animals submitted to ATZ/hypoxia followed by reoxygenation. ATZ/normoxia specifically inhibited catalase activity and caused no changes in the activities of Se-GPX, SOD, and GST throughout 33 h in normoxia (Fig. 2A). Regarding the effect of hypoxia alone, exposure of saline-injected animals to severe hypoxia did not alter the activities of total-GPX, Se-GPX, SOD, and GST. Although the ANOVA test revealed a difference of Se-GPX activity between groups, the post hoc test did not. The apparent Se-GPX increase in saline-injected animals in response to hypoxia (1.7-fold compared with controls) was not statistically significant and had a power of 38%. The combination of ATZ/hypoxia also caused no change in the activities of total-GPX, Se-GPX, SOD, and GST.

We next evaluated the effect on antioxidant enzymes of 3 h of hypoxia followed by reoxygenation in animals injected with saline or ATZ (Fig. 2B). In ATZ-injected fish, the activities of total-GPX, Se-GPX, and GST in any animal group remained unchanged upon reoxygenation. Reoxygenation increased SOD activity in saline-injected fish, whose levels were similar to ATZ-injected animals.

RESULTS

Suppression of catalase activity by ATZ. To suppress catalase activity under normoxic conditions, we injected ATZ intraperitoneally in the animals at a dose of 1 g/kg. Hepatic ATZ levels were maintained elevated up to 33 h after ATZ administration (Fig. 1A, top). Catalase-specific activity was inhibited from 72 U/mg protein (controls) to 4–6 U/mg protein at 12–33 h post-ATZ injection under normoxia (Fig. 1A, bottom). Furthermore, ATZ content and catalase activity were analyzed in animals that had been submitted to a cycle of 12 h of hypoxia followed by reoxygenation (30 min to 24 h). Hepatic ATZ levels were maintained elevated throughout the course of the hypoxia/reoxygenation cycle, as well as the suppression of catalase activity (Fig. 1B).

Activities of antioxidant enzymes: Se-GPX, total-GPX, SOD, and GST. The main objective of this study was to investigate the contribution of catalase to protection against reoxygenation-derived oxidative stress by means of in vivo enzyme inhibition with ATZ. However, hypoxia and/or ATZ may alter free radical metabolism, leading to changes in other antioxidant enzymes. Therefore, antioxidant enzyme levels from animals subjected to ATZ/normoxia or hypoxia alone were compared with data from animals submitted to ATZ/hypoxia followed by reoxygenation. ATZ/normoxia specifically inhibited catalase activity and caused no changes in the activities of Se-GPX, SOD, and GST throughout 33 h in normoxia (Fig. 2A). Regarding the effect of hypoxia alone, exposure of saline-injected animals to severe hypoxia did not alter the activities of total-GPX, Se-GPX, SOD, and GST. Although the ANOVA test revealed a difference of Se-GPX activity between groups, the post hoc test did not. The apparent Se-GPX increase in saline-injected animals in response to hypoxia (1.7-fold compared with controls) was not statistically significant and had a power of 38%. The combination of ATZ/hypoxia also caused no change in the activities of total-GPX, Se-GPX, SOD, and GST.

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Glutathione levels. The tripeptide glutathione (gamma-glutamylcysteinylglycine) has several cellular functions. For example, it acts as a soluble nonenzymatic antioxidant and as an essential cosubstrate for the function of two main groups of antioxidant enzymes, GPXs, and GSTs. Considering that the physiological concentration of glutathione influences in situ GPX activity (21, 37), we assessed its levels in all experimental groups. Total glutathione content (GSH-eq, glutathione equivalents = GSH + 2 GSSG) levels were not altered by ATZ/normoxia or by hypoxia (Fig. 3A). During reoxygenation, ANOVA test indicated an effect of ATZ in the levels of GSH-eq (GSH equivalents) in ATZ-injected animals; however, differences were not localized by the post hoc test (Fig. 3B). Oxidized glutathione (GSSG) levels remained unchanged under ATZ/normoxia, ATZ/hypoxia, and reoxygenation (Fig. 3B).

Redox imbalance and oxidative stress. The GSSG/GSH-eq ratio is a sensitive marker of redox imbalance and oxidative stress and also indicates the degree of glutathione utilization, i.e., the degree of enzyme-catalyzed GSH oxidation into GSSG in relation to the total pool of free glutathione (8, 42). The GSSG/GSH-eq ratio was not altered by ATZ/normoxia or hypoxia (Fig. 3A). At the beginning of the reoxygenation (30 min), GSSG/GSH-eq ratio in ATZ-injected animals was 34% higher (P < 0.05) than in saline-injected animals, becoming diminished in the following moments (Fig. 3B).

The levels of carbonyl groups on proteins, which are a presumptive evidence of oxidative modification by free radicals, were unaltered by the injection of ATZ or by hypoxia alone (Fig. 4A). Reoxygenation increased the levels of carbonyl protein in saline-injected fish, whose levels were similar to ATZ-injected animals. The elevated levels of carbonyl proteins indicated the presence of oxidative stress upon reoxygenation (Fig. 4B). Lipid peroxidation levels, measured as TBARS, remained unchanged upon ATZ administration, under hypoxia (Fig. 4A) or during reoxygenation (Fig. 4B).
working at their maximal potential under normoxia. Even in the face of catalase inhibition, and possibly increased concentrations of H$_2$O$_2$, these enzymes were still able to cope with such an oxidative condition and avoid signs of oxidative stress. Therefore, our results indicate that under conditions of normoxia, catalase does not play an important role in preventing oxidative stress in tilapia. However, we cannot exclude the possibility that catalase action under normoxia, together with peroxiredoxins, thioredoxins, and GPX (12), regulates H$_2$O$_2$ to levels necessary for processes of signal transduction.

The results for ATZ injections under normoxia in tilapia fish contrast with those observed in the liver of frogs Rana perezi and goldfish Carassius auratus after catalase suppression under normoxia. In these animals, catalase inhibition by ATZ caused increased oxidative damage, indicating that this enzyme is pivotal to prevent oxidative stress during normoxia (2, 4). In addition, not only catalase activity was diminished by ATZ in these animals, but also the activities of hepatic GPX in frogs (4), and GPX, GR, and G6PDH in goldfish (2). In the present study, the maintenance of diverse endogenous antioxidants in ATZ-injected tilapias under normoxia facilitated the observation of the changes that occurred upon the reoxygenation-derived oxidative stress, which was the purpose of this study.

Effect of hypoxia alone. Tilapia fish injected with saline and submitted to severe hypoxia showed no statistically significant changes in the levels of antioxidant enzymes, glutathione status, and oxidative stress markers. Normally, during lower oxygen supply (anoxia/hypoxia), there is a general reduction in the rate of protein turnover or metabolism in hypoxia-tolerant animals (24, 26). The maintenance of the levels of tilapia antioxidants despite the expected general lowering of enzymes during hypoxia indicates that the antioxidant system plays an important role in oxygen availability disturbances. Despite this reduction in protein turnover during anoxia or hypoxia, specific genes show a higher rate of transcription and translation (26, 49, 55). This selective increase in gene expression is probably important for the animal’s resistance to the oxygen restriction and/or to the reoxygenation-derived oxidative stress. Other studies showed higher activity or expression of antioxidant enzymes in response to oxygen restriction, particularly Se-GPX, which would be important in limiting oxidative stress derived from abrupt elevations in oxygen availability/consumption [a mechanism named “preparation for oxidative stress” (13, 23)]. For instance, anoxia/hypoxia exposure induced an increase in activity or expression of Se-GPX in the freshwater snails, Biomphalaria tenagophila (13), in frogs, Rana pipiens (22), in goldfish, C. auratus (32), in carp (31), in turtles, Chrysemys picta marginata (52), and in Pacific oysters, Crassostrea gigas (11).

The mechanisms regulating antioxidant enzymes in hypoxia-tolerant species are still poorly understood. It is possible that they are associated with the adjustment to anaerobic metabolism. Fish submitted to anoxia/hypoxia exposure show the same responses of well-known genes seen in other animals (29). The extreme anoxia-tolerant crucian carp, Carassius carassius, shows increased amounts of hypoxia-inducible factor 1 (HIF-1, a transcription factor involved in the response to oxygen levels) protein in liver, gills, and heart in response to hypoxia (46). HIF-1 is involved in the induction of erythropoiesis in various vertebrates. The air-breathing African catfish, Clarias gariepinus, submitted to asphyxia shows increased hematocrit and hemoglobin content (56). In the fish medaka, Oryzias latipes, hypoxia causes upregulation of genes that are targets of HIF-1 (24). In addition to HIF-1, nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) has been investigated in recent studies as a potential regulator of antioxidant enzymes in freeze-tolerance turtles (25) and dehydration-resistant anurans (35). The stress of freezing in painted turtles, Chrysemys picta bellii, and of dehydration in African clawed frogs, Xenopus laevis, more frequently causes increased levels of GST isoenzymes than suppression of their levels, in an organ-specific manner. Some of these effects may be under regulation of Nrf2 transcription factor (25, 35).

The metabolic adjustments that improve the antioxidant system are especially present in animals that naturally experience and tolerate a wide variation in oxygen availability. Different from goldfish (32) and tilapia, which show an increase or a maintenance of antioxidant parameters during hypoxia, the less hypoxia-tolerant common carp Cyprinus carpio (54, 57) shows some similarity to what happens with reperfusion-sensitive rodents. This fish shows increased oxidative stress during reoxygenation after a period of hypoxia. Its hepatic activities of catalase, GR, GST, SOD, and G6PDH remain unchanged, and Se-GPX activity decreases in response to hypoxia (31). In the case of the Atlantic cod (Gadus morhua), which presents an even lower tolerance to reduced oxygen supply, hypoxia causes a significant downregulation of transcript levels of hepatic GPX and SOD (38).

Effect of hypoxia plus ATZ. Various authors consider hypoxia exposure as a situation in which there is a higher mitochondrial ROS production (6, 7, 17, 34). This has been reported in rodent neurons (1) and in rat muscle (9, 63). Hypoxic exposure during 48 h decreases the capacity for oxidative phosphorylation in mouse skeletal muscle and increases mitochondrial markers of oxidative stress (33). Because ATZ is known to increase H$_2$O$_2$ content, a higher production of ROS upon hypoxia would be exacerbated in ATZ-injected animals, mainly regarding the levels of H$_2$O$_2$. Surprisingly, ATZ-injected tilapia fish showed unchanged signs of hepatic redox balance and oxidative stress in response to hypoxia, despite the expected higher levels of H$_2$O$_2$.

The absence of statistically significant changes in the antioxidant system during hypoxia in tilapia fish indicates that oxygen restriction does not severely affect its free radical physiology and that catalase is not essential to maintain its redox balance under such a condition. Considering that other studies show Se-GPX and GSH as very important antioxidants in controlling oxidative stress due to an excess of H$_2$O$_2$, the maintenance of their levels in tilapia during hypoxia indicates that they contribute to avoid redox imbalance. Specifically, Se-GPX activity is measured in vitro at supraphysiological levels of its substrate peroxide (0.2 mM) and the tissue concentration of H$_2$O$_2$ is several times lower (nanomolar range). Therefore, the in vivo activity—decomposition of peroxide—is dependent on substrate availability (37) and, despite no changes in Se-GPX activity and GSH levels, they would contribute toward minimizing oxidative stress upon hypoxia. Other antioxidants/factors may also be relevant in the control of the effects of H$_2$O$_2$ overproduction, for example, peroxiredoxins and glutathionylation, redox parameters (41), whose investigations are incipient in comparative animal physiology.
Effect of reoxygenation plus ATZ. Reoxygenation resulted in relatively high SOD activity, which indicates that during this period, ROS formation is greater than under hypoxia. This rationale is in accordance with data from the literature, because a positive relationship between SOD activity and ROS production has been demonstrated in various studies (15, 16, 36, 50). Lower oxygen saturation, which has been reported to elevate ROS levels, also promotes increases in liver SOD activity in the estuarine fish Leistostomus xanthurus (10) and in the freshwater fish Perccottus glenii (30). The data of carbonyl protein corroborate the explanation that there would be an augmented generation of ROS during reoxygenation. Carbonyl protein levels became elevated upon reoxygenation, which confirms previous findings that several hypoxia-tolerant species survive cycles of reoxygenation-derived oxidative stress, maintaining oxidative damages at a manageable level. Increased levels of oxidative damage in the anoxia-tolerant goldfish during post-anoxic reoxygenation are also considered physiological, because they are adapted to this kind of stress in nature (32).

At the beginning of the reoxygenation period, the level of GSSG/GSH-eq ratio was higher in ATZ-injected tilapias than in saline-injected ones, indicating that the animals with depleted catalase showed an increase in the steady-state concentration of GSSG. The increased GSSG/GSH-eq ratio indicates that this is a condition of redox imbalance associated with elevated oxidation of GSH into GSSG. The higher action of the GSH-dependent system indicates that catalase plays an important role during reoxygenation in this species. The increased effects of ROS during hypoxia-recovery associated with catalase suppression in tilapia are probably the result of a rise in steady-state H2O2 concentrations, due to increased production of this oxidant caused by reoxygenation itself and less H2O2 decomposition due to catalase depletion. It is important to consider that H2O2 is a product of SOD reaction; therefore, a rise in H2O2 levels would be expected in response to the augmented activity of SOD during reoxygenation. Although SOD activity was similar in both saline- and ATZ-injected fish and its effect on H2O2 production should be the same, GSSG/GSH-eq ratio became higher specifically in ATZ-injected animals. This information reinforces that the difference between the two groups was a response of the catalase suppression.

In contrast to the expectations based on data from other animals that experienced catalase inhibition by ATZ, catalase-suppressed tilapia showed unchanged levels of the oxidative stress markers, carbonyl protein, and TBARS during reoxygenation compared with saline-injected animals. A factor that possibly contributed to minimize oxidative injuries in hepatic tilapia tissue during reoxygenation was the observed high availability of GSH-eq. In both saline- and ATZ-injected tilapia fish, the concentration of GSH remained unchanged during reoxygenation despite an expected fall because of the increase of its use (47). This suggests that the enzymes responsible for GSH biosynthesis or the event of deglutathionylation were maintained or activated. We speculate that the initial ROS detoxification events by the tilapia antioxidant system may intensely use GSH to ensure return to normality. In red-eared turtles, part of their impressive resistance to anoxia-reoxygenation cycles is explained by a high constitutive concentration of GSH in their tissues (60). Interestingly, hepatic GSH levels in tilapia are similar to those in turtle liver.

In nature, several conditions are known to induce a long-term production of ROS in fish, such as variations in oxygen and temperature, and, consequently, redox imbalance and oxidative stress. In this work, we used a situation that mimics an environmental drastic event—acute reoxygenation. The results indicate that this species is very resistant to acute environmental changes and that catalase in these situations may be partly compensated by other antioxidant components. Actually, ATZ could also be used to investigate some longer-lasting phenomena, such as the role of catalase in detoxifying xenobiotics derived from contamination in aquatic ecosystems.

In conclusion, our experimental protocol using the hypoxia-tolerant tilapia fish and combining suppression of catalase activity with a hypoxia/reoxygenation cycle—promoting hepatic oxidative stress in this species—indicated that pharmacological inhibition of catalase causes redox imbalance during the early stages of reoxygenation, which is not sufficient to evolve to oxidative stress (i.e., further oxidative damage, determined by lipid peroxidation or protein oxidation) typical of the episode.

Perspectives and Significance

Taking into consideration that reoxygenation is well known to be an exceptional condition of high ROS production in many animals, which mimics the ischemia-reperfusion event, our data indicate that catalase in tilapia fish is a relevant antioxidant when ROS production in liver is supposed to reach high levels. Further investigations on the effects of catalase suppression on the activities of peroxiredoxins and enzymes involved in deglutathionylation might help in the understanding of the orchestrated mechanisms involved in the control of redox balance in tilapia fish under hypoxia/reoxygenation. Similar to gene knockout studies, drug-mediated enzyme inhibition studies associated with different oxygen concentrations (as used in this study) may help to clarify the role of each constituent of the antioxidant system against hypoxia/reoxygenation-mediated oxidative stress. Such studies could involve the use not only of ATZ, but also of inhibitors of GSH biosynthesis and of GR and GST activities. The present study demonstrates the feasibility and usefulness of such approaches.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


